STUDIES ON SERUM DEPENDENT *IN VITRO* MONOCYTE FUNCTION IN RHEUMATIC DISEASES

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Lund 1980

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Abstract

A method for the study of serum dependent phagocytosis was developed. Human monocytes were cultivated for 2 hours in a medium with 15 % serum whereafter 10^7 heat-killed yeast cells (YC) were added for phagocytosis.

Low phagocytic activity (PA) was found in 51 % of 43 SLE-sera, in 16 % of 57 RA-sera, in 22 % of 18 sera from patients with SLE-like syndromes but in none of 46 sera from patients with other inflammatory rheumatic disorders. Low PA was associated with hypocomplementaemia, presence of cryoglobulins in SLE-sera, high titres of anti-nDNA antibodies and, in the SLE-patients, with high clinical disease activity and evidence of arthritis, rash and nephritis.

To investigate possible mechanisms behind impaired YC phagocytosis (YCP) 2 types of experiments were performed: 1) Immune complex analogues were added to monocyte cultures in normal serum. The results showed that addition both of heat aggregated IgG (HAG) and of cryoglobulins isolated from patient sera could reduce PA. 2) *Pre*-opsonized YC were added to monocyte cultures with HAG or with SLE-serum. Phagocytosis was then unaffected, whereas, in parallel cultures, phagocytosis of not-preopsonized YC was impaired.

Other *in vitro* monocyte functions were studied in sera from patients with SLE. Glass adherence and spreading by monocytes was decreased in presence of sera from patients with active SLE. Bacterial killing by normal monocytes was impaired in 8 of 14 randomly chosen SLE-sera.

These findings gave evidence for the possibility that impairment of YCP found in many SLE-sera may be related to the presence of immune complexes, possibly due to depletion of complement components. It was suggested that the impairment of glass adherence and spreading demonstrated in such sera may have a similar mechanism.

Key words
Monocyte, macrophage, phagocytosis, yeast cells, glass adherence, spreading, bacterial killing, cryoglobulins, heat aggregated IgG, rheumatic diseases, systemic lupus erythematosus

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This thesis is based on the following publications:

I Björn Svensson: Methodological studies of monocyte yeast cell phagocytosis. Scand J Rheumatology, Suppl 31:5, 1980.

II Björn Svensson: Occurrence of deficient monocyte yeast cell phagocytosis in presence of rheumatic sera. Scand J Rheumatology, Suppl 31:21, 1980.

III Björn Svensson: Monocyte *in vitro* function in systemic lupus erythematosus (SLE). I. A clinical and immunological study. Scand J Rheumatology, Suppl 31:29, 1980.

IV Björn Svensson & Gunnar Sturfelt: Monocyte *in vitro* function in systemic lupus erythematosus (SLE). II. Glass adherence and spreading in presence of SLE-sera. Scand J Rheumatology, Suppl 31:43, 1980.

V Ola Nived, Håkan Odeberg & Björn Svensson: Monocyte *in vitro* function in systemic lupus erythematosus (SLE). III. Bactericidal activity in presence of SLE-sera. Scand J Rheumatology, Suppl 31:53, 1980.

VI Björn Svensson, Renée Norberg & Rigmor Torstensson: Effects of cryoglobulins and aggregated IgG on *in vitro* monocyte phagocytosis. Scand J Rheumatology, Suppl 31:57, 1980.

The publications are referred to by their roman numericals.

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INTRODUCTION

Mononuclear phagocytes (blood monocytes and tissue macrophages) and polymorphonuclear leucocytes are cells specialized in host defence activities. Their common functional hall-mark is phagocytosis. This study is confined to the function of monocytes in rheumatic diseases.

The mononuclear phagocytic system

The mononuclear phagocytes constitute the so-called mononuclear phagocytic system as proposed by van Furth et al. (1972). The mononuclear phagocytes arise in the bone-marrow from stem cells, which they possibly share with the granulocytes (Brandt 1974, Bradley & Metcalf 1966). The stem cells develop into monoblasts, then to promonocytes and leave the bone-marrow as monocytes (van Furth 1970). The blood monocytes transit in the peripheral vasculature as circulating and marginating (ratio 1/3.5) cells (Meuret & Hoffman 1973) for about 30 hours (van Furth & Cohn 1968). After that time they migrate through the vessel walls into tissues throughout the body. There they develop into functionally heterogenous populations (Walker 1976) of tissue macrophages which may live for months (van Furth 1970). They do not recirculate (Territo & Cline 1975) and mitoses are infrequent unless the macrophage is challenged by inflammatory or other stimuli (Spector 1977).

Activities of mononuclear phagocytes

Mononuclear phagocytes have ever since the end of the last century been recognized as essential parts of the host defence against microbes (Metschnikoff 1883, Metsch-

nikoff 1892). A major activity of mononuclear phagocytes in 'unchallenged' conditions is the non-immune clearing of damaged cells, of degraded proteins and of fibrin products in wound repair (scavenger activities).

In recent years evidence has accumulated that these cells have important roles in the immune response to most kinds of antigenic challenge.

The mononuclear phagocytes are essential for a normal antigen processing (reviewed by Snyderman & McCarty 1978). They have capacity to rapidly localize and destroy antigenic material thus protecting the host from antigenic overload. Antigenic material may also be retained in the macrophage and, in a modified form, be relocalized on the macrophage surface, possibly in complex with so-called Ia-molecules functioning as the antigen-recognition molecule on the macrophage (Niederhuber 1978).

The macrophages are involved in immunoregulation also by means of secretory activities, e.g. secretion of a lymphocyte-activating factor (LAF), which may act as a substitute for macrophages in MLC-reactions (Bach et al. 1970). Furthermore, mononuclear phagocytes secrete substances which may modify the immune response, e.g. complement components (Bentley et al. 1978), prostaglandins, interferon, cyclic AMP (Allison 1978), collagenases and other proteases (Snyderman & McCarty 1978). In addition, mononuclear phagocytes have a hydrogen-peroxide-generating system of importance for bacterial killing (Territo & Cline 1975, Cohn 1978).

Macrophages have long been recognized as essential for an efficient elimination of various microbes, e.g. of facultative intracellular bacteria (Hahn 1975), parasites (Mauel et al. 1975) and viruses (Silverstein 1975). In addition, the findings of generation and release of oxygen-intermediates suggest that macrophages may be supplementary to granulocytes in the elimination also of other microbes. Thus, in pronounced neutropenia infections might be less severe if monocytosis is present, e.g. in cyclic neutropenia (Brandt et al. 1975). Macro-

phages have been found to be active in the destruction of tumour cells and are believed to play a role in tumour surveillance (Keller 1977). Mononuclear phagocytes may also be involved in the clearing of circulating immune complexes (Haakenstad & Mannik 1974, Frank et al. 1979) and in the host response to various microbial and non-microbial antigens in delayed hypersensitivity reactions (Bloom & Bennett 1970).

Functional characteristics of mononuclear phagocytes

The hall-mark of mononuclear phagocyte function is phagocytosis. Phagocytosis may be described as endocytosis of solid particles of varying size. Pinocytosis denotes the endocytosis of soluble molecules and may be working through mechanisms other than those responsible for phagocytosis (for review see Hed 1979).

During phagocytosis, cytoplasmic extensions, so-called pseudopods,surround the particle which subsequently becomes interiorized into the cell in a phagocytic vacuole. In a study of the mechanisms of phagocytosis Griffin et al. (1975) present data indicating that 'the step by step interaction of membrane receptors with particle-bound ligands may result in a zipper-like movement of the membrane around the particle'. This movement of pseudopods around the particle is believed to be promoted by the action of cytoplasmic filaments. Thus, cytoplasmic actin is said to polymerize and form a gel which will be contracted by cytoplasmic myosin (see Hed 1979).

The mononuclear phagocytes may recognize the material to be phagocytosed unspecifically, e.g. non-pathogenic bacteria, carbon-particles or latex-particles (Walters and Papadimitriou 1978). However, the recognition of most pathogenes requires opsonization (Wilkinson 1976), i.e. coating of the microbes with serum opsonins which was first described in 1903 by Wright & Douglas (1903). The best defined serum opsonins are IgG-antibody and the complement component C3. By means of its cell surface receptors for these opsonins the macro-

phage can recognize, attach and eventually interiorize opsonized particles. These receptors may be involved in the binding and/or uptake also of soluble immune complexes (Haakenstad & Mannik 1974).

The mononuclear phagocytes are capable of locomotion. In response to chemoattractants, such as the complement derived C5a, spreading increases and the cell 'crawls' against the concentration gradient (Stossel 1978). Other stimuli, e.g. immune complexes or lymphokines from sensitized T-lymphocytes may activate the macrophage leading to a secretory response (Allison et al. 1978).

The mononuclear phagocytes perform most of these functions when they have been activated by different sorts of stimulation. Activation (North 1978) is morphologically expressed as increase in cell size, development of a ruffled cell membrane surrounding an abundant, richly granulated cytoplasm. The activated mononuclear phagocyte shows increases of cell surface stickiness, oxygen-consumption, enzyme production and phagocytic activity (Cohn 1978).

Functional disorders of mononuclear phagocytes

Clinically relevant impaired function of mononuclear phagocytes has previously been reported. Thus, as reviewed by Territo and Cline (1977) deficient monocyte chemotaxis has been described in the Chediak-Higashi syndrome, in mucocutaneous candidiasis and in patients with cancer. Impaired microbial killing has been found in chronic granulomatous disease, in malignant lymphoma and in patients on corticosteroid therapy.

Monocyte function in inflammatory rheumatic diseases

The etiology of the inflammatory rheumatic diseases is largely unclear (Quimby & Schwartz 1978). In diseases like systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) evidence of immune dysfunction is almost always demonstrable. For instance, in SLE, auto-antibodies (for review see Glass & Schur 1977), circulating

immune complexes (Lambert & Casali 1978), deposition of immune complexes (Koffler et al. 1967), hypocomplementaemia (Schur 1975), depressed immune reactions (Lavalle et al. 1978, Andrianakos et al. 1977) and suppressor-cell dysfunction (Sagawa & Abdou 1978) are well recognized.

As mononuclear phagocytes are essential in most aspects of immunity, a dysfunction of these cells might conceivably contribute to the pathogenesis of inflammatory rheumatic diseases and might also be more or less responsible for the increased susceptibility to infections and the occurrence of lymphoreticular malignancies described in SLE (Green et al. 1978) and Sjögren's syndrome (Anderson & Talal 1972).

To investigate whether macrophage function is impaired in SLE a pilot study was performed in 1973 (Svensson & Hedberg 1973). The yeast cell phagocytosis (YCP) by buffy-coat derived glass activated human macrophages was studied in 7 patients with active SLE. The results showed that YCP by SLE-macrophages was significantly lower than that of simultaneously cultured macrophages from healthy volunteers. In a later study, published in 1975 (Svensson 1975), this macrophage dysfunction proved to be mainly serum dependent as low YCP was also obtained with normal macrophages in SLE-sera but not with SLE-macrophages in normal sera.

These studies support the idea that serum dependent macrophage dysfunction might exist in SLE. However, the information obtained in these studies was based on a small number of selected patients with SLE. Therefore, an extended study of <u>in vitro</u> monocyte phagocytosis performed on a greater number of patients with SLE was called for. Furthermore, it was of interest to extend these studies to patients with other inflammatory rheumatic diseases. The method used in the above-mentioned studies had some drawbacks, which made it inconvenient for such a study. The incubation (4 days) was too long. Thus, the monocytes developed into large activated cells with a high phagocytic activity. At the time of phago-

cytosis (at 4 days) many macrophages ingested so many yeast cells that these could not be counted. Furthermore, the cell population used contained a considerable number of granulocytes.

In a study of the possible roles of mononuclear phagocytes in the pathogenesis of inflammatory rheumatic diseases, the influence of rheumatic sera on monocyte activities other than phagocytosis, e.g. glass adherence, spreading and bacterial killing, would certainly also be of interest.

Aims of the present study

On the basis of the considerations given above the aims of the present investigation were the following:

1) To develop a method for the study of serum dependent monocyte yeast cell phagocytosis suitable for clinical investigations.

2) To apply this method to a study of the occurrence and clinical relevance of impaired monocyte yeast cell phagocytosis in some inflammatory rheumatic diseases.

3) To investigate possible mehcanisms behind impaired monocyte yeast cell phagocytosis.

4) To study monocyte *in vitro* glass adherence and spreading in presence of sera from patients with SLE.

5) To study monocyte bacterial killing in presence of sera from patients with SLE.

THE PRESENT INVESTIGATION

1. Methods used for measuring monocyte function

Cell separation and cultivation (I)

Monocytes were obtained from the peripheral blood of healthy donors (members of the hospital staff). The blood was defibrinated by gentle shaking in a flask with glass beads.

The blood cells were separated by the metrizoate-polysucrose (Isopaque-Ficoll) method described by Bøyum (1968). The monocyte-rich cell layer was suspended in a medium with serum in Parker 199 (National Bacteriological Laboratory, Stockholm) and added to a glass chamber (inner diameter 13 mm and height 7.5 mm) as described by Nordqvist and Rorsman (1970). After incubation at 37°C, the monocytes formed a glass adherent monolayer on the bottom of the chamber.

Yeast cell phagocytosis and glass adherence (I)

Procedure. Yeast cells (YC) (Baker's yeast, Saccaromyces cerevisiae) were suspended in saline and killed by heating at 100°C for 30 minutes in a water-bath as described by Brandt (1967).

At the end of the incubation of the monocytes, 0.5 ml of the medium was replaced by an equal volume of a suspension of YC in 0.9 per cent saline. The cultures were then re-incubated at 37°C in order to allow phagocytosis to occur.

The incubation time before phagocytosis, the serum concentration in the medium, the concentration of YC and the time of re-incubation with YC were varied as

described below. The results, discussed below, suggested that a suitable 'basic' model would be to incubate the monocytes in 15 per cent serum for 2 hours before a further incubation for 30 minutes with 10^7 YC. In the following,this system has been used unless otherwise stated.

Phagocytic activity. The phagocytic activity (PA) was expressed as the median YC uptake per monocyte in 100 monocytes. The counting was made in 16 pre-determined fields of vision representing both central and peripheral areas. Double determinations were always done (counting in two parallel cultures).

Glass adherence. In light microscopy, the total number of glass adherent mononuclear cells (GAC) per culture was calculated from a count of all cells in the 16 representative fields of vision described above and extrapolated to the total bottom area of the chamber.

Recovery. Identification of monocytes in the cell suspension was based on the esterase-staining method of Yam and Crosby (1971). Recovery was expressed as the number of glass adherent cells per 100 monocytes added. In 12 different experiments the mean recovery was 81 per cent, range 47-115 per cent.

Influence on PA of initial (before phagocytosis) serum concentration in the medium. PA was very low if serum was absent from the medium. In 23 donors of cells and serum where the monocytes were incubated in 15 per cent autologous serum, the mean PA was 9.96 $\pm$ SD 1.56. Increasing the initial serum concentration in the medium from 15 to 50 or 100 per cent resulted in some increase of PA while lowering the initial serum concentrations below 15 per cent resulted in a reduction of PA that was obvious at 6 per cent and very marked below this concentration.

Influence of the incubation time on PA and GAC. Shorter incubation times than 2 hours resulted in low GAC and therefore PA-values were not determined. Increasing the incubation time from 2 hours to 24 hours did not change either GAC or PA. Prolonged incubation to 3 or more

days, however, highly increased PA while the changes in GAC showed no uniform trend.

Influence of adding YC on GAC. The addition of YC reduced GAC significantly. Thus, the results of 16 experiments showed that the median GAC was 90 x 10^3 before adding YC and 65 x 10^3 after adding YC ($p<0.01$).

Influence of YC incubation time on PA and GAC. Incubation with YC for less than 30 minutes gave a lower PA and a higher GAC. An increase of the YC incubation time to 60 minutes tended to reduce GAC but produced little change of PA.

Monocyte/YC ratio and PA. GAC after yeast cell phagocytosis (YCP) from the 23 experiments mentioned above varied between 15 x 10^3 and 105 x 10^3. Addition of a constant number of 10^7 YC gave an actual monocyte/YC ratio between about 1/100 and 1/800. Variation of the ratio within this range did not affect PA.

Distribution of PA in 'normal' sera. The distribution of PA (mean of duplicate determinations) was studied in 37 sera from healthy donors (23 autologous and 14 homologous). The mean of this population was 9.99, the median 10 and the range 7-13. The distribution was approximately normal. As the sample was rather small it cannot be excluded that small deviating subpopulations might exist within the 'normals'.

Experimental sources of variation of PA. In order to get some information of experimental sources of variation of PA two series of experiments have been performed. In the statistical analysis - by analysis of variance (Snedecor 1955) - it has provisionally been assumed that the PA-values are normally distributed.

Inter- and intraindividual variations of PA for autologous sera were determined (in duplicate as usual) in repeated experiments on sera from 4 donors. Fresh serum was used at each experiment. The number of experiments was 20, 12, 10 and 6 (total 48) for the different donors.

In another series of experiments the variation between PA for the autologous serum and for an homolo-

gous serum (both in duplicate) was determined. The number of homologous sera tested was 14.

The results of these two studies of variation of PA are condensed in Table I. The standard deviation of PA for the same autologous serum determined at the same experiment was 0.54. When determinations were made at different experimental occasions the standard deviation was considerably increased (SD 1.80, $p<0.01$). The variation between different autologous sera (at different experiments) was still larger (SD 2.71) but did not reach significance.

When PA for homologous and autologous sera were determined in simultaneous experiments the variation between them was significantly larger (SD 1.57, $p<0.01$) than that for PA of identical sera.

The method chosen for evaluation of PA for test sera. In order to avoid the inter-experimental error found, PA of a test serum was always compared with PA of the autologous serum (from the monocyte donor). The phagocytic capacity of monocytes in test serum was expressed as the difference between PA of test serum and PA of the autologous serum (both PA calculated as the mean of duplicate determinations).

If it is assumed that PA of 'normal' sera are normally distributed, population limits can be calculated for the differences found between PA of homologous serum and PA of autologous serum from the 14 experiments mentioned above. The 95 per cent tolerance limits (or confidence limits) of this standard population of differences are -4.03 and +2.95.

Spreading (IV)

Spreading was determined according to the method described by Territo and Cline (1977). Thus, the longest diameter of the cytoplasm of 100 glass adherent monocytes per preparation was measured with a microscale in a phase-contrast microscope using an oil-immersion objective lens (12.5 x 100). The cells were counted in sequential fields in a standardized manner. Only cells without con-

TABLE I.Variation of PA in different experimental set-ups. The size of variaton is expressed as the standard deviation.

		Autologous serum		Homologous serum
		same donor	different donors (4)[x)]	different donors (14)[x)]
Autologous serum	within experiments	0.54 n = 48 df= 48		1.57 n = 14 df= 13
	between experiments	1.80 n = 48 df= 44	2.71 n = 48 df= 3	

x) number of donors

n = number of experiments

df = degrees of freedom

tact with other cells were counted.

In the present study, cells with a diameter of ≥15 μ were considered spread.

Bacterial killing (V)

1) A cell suspension was prepared as described above.

2) Staphylococcus aureus (strain 502 A) was used as test organism. An overnight culture was washed and suspended in Parker 199. A final bacterial concentration of approximately 2×10^7 colony forming units/ml was achieved.

3) The bacterial killing experiments were performed as a modification of the Maaloe-technique (Maaloe 1946). Approximately 4×10^6 Staphylococcus aureus were added to 0.8 ml of the cell suspension containing about 6×10^6 mononuclear cells of which 15-25 per cent were monocytes. The incubations were performed at 37°C during rotation at 20 rpm in a horizontal position. Aliquots of 20 μl were removed at 120 minutes and diluted in 8 ml distilled water containing 0.01 per cent bovine serum albumin. Viable bacteria were determined by colony counting.

A monocyte bactericidal index (MBI) was calculated according to the leucocyte bactericidal index of Hoffman and Bullock (1973):

$$MBI = \frac{\log \%\ \text{viable}^{120'}_{\text{control}} - \log \%\ \text{viable}^{120'}_{\text{unknown}}}{\text{normal 'between-subject' SD}^{120'}}$$

The normal 'between-subject' variance was determined by assaying monocyte bacterial killing in 7 control sera on the same occasion. An MBI of -2 or lower indicates significantly reduced bactericidal activity ($p<0.05$).

Comments

For in vitro studies macrophages may be easily obtained from various organs of laboratory animals. However, in humans it is difficult to obtain normal tissue macro-

phages. Therefore, the blood monocyte is, with few exceptions, the only human mononuclear phagocyte that has been used for *in vitro* studies (Gadeberg et al. 1979). Previously, this cell was difficult to isolate and concentrate but in recent years several techniques have been developed to overcome these problems (see Gadeberg et al. 1979). Among these, the method described by Bøyum in 1968 (Bøyum 1968) was one of the first to appear, is rapid and simple and has therefore been used in the present study.

This investigation intended to study monocyte *in vitro* functions in presence of normal and pathological sera. Yeast cells (YC) as phagocytic material have previously been used to detect serum dependent impairment of phagocytosis in systems both with granulocytes (Quie 1972) and with monocytes (Svensson 1975). YC are well suited to such studies as they are only poorly phagocytosed in absence of serum (Rosenfeld et al. 1977). Furthermore, available evidence (Brandt 1967, Hed & Stendahl 1978, Hed 1979, Yamamura & Valdimarsson 1978) indicates that antibodies to YC are infrequent in or absent from human sera. Therefore, it is not likely that YC antibodies are an important source of variation in the determination of phagocytic activity. In addition, YC are easy to prepare and store. They stain distinctly which makes measurement of phagocytosis under the microscope easy.

From the observations in the microscope it was not always possible to decide whether the YC were completely or partly ingested or only attached to the cell walls. Previous observations using the same system (Rydgren et al. 1978) have shown that most of the YC really are ingested. This is in agreement with the recent findings of Hed (1979) that almost all cell-associated YC were interiorized after incubation with polymorphonuclear granulocytes for 30 minutes.

In order to identify the monocytes in the cell suspension, the esterase-staining procedure described by Yam and Crosby (1971) was used. By this method it was

shown that about 20 per cent of the cells in the cell suspension were monocytes which is in agreement with the findings of others (Gadeberg et al. 1979). Most of these cells, about 80 per cent, could be recovered as glass adherent monocytes in the culture chambers. However, the possibility that the monocyte populations obtained by the present method might not be entirely representative of the in vivo population of circulating monocytes cannot be ruled out.

The results of varying the serum concentration, the cultivation time and the YC incubation time indicated that a sufficient span for measuring subnormal phagocytosis is obtained if the monocytes are incubated in 15 per cent serum for 120 minutes followed by a further incubation with 10^7 YC for 30 minutes. This procedure was later used for clinical application (II and III).

If, instead, the serum concentration in the medium had been increased, the sensitivity of the method for detecting deficiencies of serum factors that are required for phagocytosis might have been decreased. On the other hand, increase of serum concentrations might instead augment the sensitivity of the method for detecting inhibitors of phagocytosis.

A rather wide variation of GAC could be tolerated since PA was not affected by a variation of the monocyte/YC ratio between 1/100 - 1/800.

The bacterial killing assay differed from the other monocyte function studies in that the monocytes were kept in suspension by rotation of the test tubes (<10 % of monocytes were glass adherent).

The studies of glass adherence and spreading were performed essentially as previously described (Territo & Cline 1977). However, the previous studies did not concern the influence of serum factors on the glass adherence or spreading of normal human monocytes.

Cells with a diameter of $\geq 15\mu$ were considered spread since they usually had broad and elongated lamellopodia. Cells with a diameter of $<15\mu$ had a roundish appearance and were considered non-spread. This

arbitrary definition of spreading seems to be generally in agreement with that of Rajaraman et al. (1977) while other authors (Taylor 1961, Bianco et al. 1976, Territo & Cline 1977) have not used such definitions.

The PA of 37 'normal' sera showed an approximately normal distribution and a range of 7-13.

There was a significant inter-experimental error of the determination of PA. The possible causes of this variation have not been explored. To eliminate this error the phagocytic capacity of monocytes in a test serum was expressed as the difference between PA of the test serum and PA of the autologous serum (from the donor of monocytes), which thus served as control serum. This difference is denoted $\triangle$PA t-c.

Furthermore, there was a significant variation of PA between 'normal' sera. It is apparent that it would have been possible to eliminate this variation if a standard pool of normal sera had been used as control serum instead of autologous serum. This possible improvement of the method was, however, apprehended too late to be introduced into the study.

The $\triangle$PA t-c of 14 homologous sera served as a 'standard population' for reference. The lower 95 per cent tolerance limit of this population was -4.03 (the upper limit is not of interest in this study). However, this limit can only be regarded as provisional as long as normal distribution of PA has not been shown for a larger population of normal sera than that of this study (37 sera). Accordingly the limit -4.03 has not been used statistically for division of $\triangle$PA t-c into 'normals' and 'subnormals'. In comments to experimental results values of $\triangle$PA t-c below -4.03 are called 'low' for the purpose of orientation.

Owing to the fact that normal distribution of PA has not been sufficiently substantiated,non-parametric statistical methods have been used in the following studies II and III.

2. Effects of rheumatic sera on monocyte function

Clinical material (II and III)

Included in the study were 164 patients who were in-patients or out-patients at the Department of Rheumatology, University Hospital, Lund, Sweden. They may be regarded as 'consecutive' cases as all patients with a particular diagnosis during a certain period of time were included in the study. They were divided into the following 4 diagnostic groups:

			N
SLE-group:	Systemic lupus erythematosus		43
CTD-group:	Other multisystem inflammatory connective tissue diseases		34
	SLE-like syndromes	18	
	Progressive systemic sclerosis	5	
	Dermato-polymyositis	4	
	Polyarteritis nodosa	5	
	Wegener's granulomatosis	1	
	Behçet's disease	1	
RA-group :	Rheumatoid arthritis		57
IJD-group:	Other chronic inflammatory joint diseases		30
	Psoriatic arthropathy	15	
	Ankylosing spondylitis	7	
	Juvenile chronic arthritis	8	

SLE was diagnosed on basis of principles of multisystem disease, serologic positivity and lack of other diagnosis as described by Fries and Holman (1975). In addition, the SLE-patients had 4 or more of the criteria for classification of SLE proposed by the American Rheumatism Association (ARA)(Cohen et al. 1971) although the ANA-test was substituted for the LE-cell-test. The patients with SLE-like syndromes had an inflammatory multisystem connective tissue disease suggestive of SLE but only full-filling 3 of the above-mentioned ARA-criteria.

The RA-patients had classical or definite rheumatoid arthritis according to criteria set by ARA (Ropes et al. 1959).

In addition to some of these diagnoses,18 patients also had Sjögren's syndrome (SS) including keratocon-

junctivitis sicca and/or xerostomy and 14 patients had features of 'mixed connective tissue disease' (MCTD) defined rather widely as a multisystem connective tissue disease with speckled ANA and a high titre of antibodies to ribonucleoprotein (RNP).

For comparisons of immunological data with the level of activity of the inflammatory process in SLE as expressed by clinical symptoms and signs, a clinical activity index (CA) free from serological data was constructed: For each patient the general condition and current focal symptoms and signs were recorded and weighted on a 3-graded scale. A severely affected general condition or a pronounced inflammatory organ involvement was assigned 2 points while 1 point was assigned to a moderately affected general condition or a moderate, ongoing inflammatory process. Inactive processes were accordingly given no points. The clinical disease activity (CA) was expressed as the sum of points for each patient and was made without knowledge of the outcome of the phagocytosis test or the serological investigations. More than 2 points meant moderate or high clinical disease activity while 2 points or less meant low or inactive disease.

For the SLE-patients, the prevalence of ARA-criteria was also recorded.

At the same time as serum was obtained for the monocyte function studies, serum and plasma were also saved for the following serological investigations:

Complement components, Clq, Cls, C4, C3, factor B, properdin and C5 were determined by electroimmunoassay (Laurell et al. 1978, Sjöholm 1975) (kindly performed by Dr. Anders Sjöholm).

Serum cryoglobulins as described in I.

Clq-binding immune complexes. The Clq-binding activity (ClqBA-test) according to Zubler et al. (1976) was used (kindly performed by Dr. Renée Norberg and Dr. Anders Sjöholm).

Antinuclear antibodies (ANA) were assayed by an indirect immunofluorescence test (Jonsson & Norberg 1978), antibodies to native DNA (anti-nDNA) by the Crithidia

luciliae-test (Aarden et al. 1975) and antibodies to extractable nuclear antigens (anti-RNP and anti-Sm) by the passive haemagglutination technique (Jonsson & Norberg 1978). All antinuclear antibody tests were kindly performed by Dr. Renée Norberg.

Orosomucoid, CRP, IgG (Laurell 1972) and rheumatoid factor (Ball 1950) were also determined in some sera.

When considered relevant, further clinical and laboratory data were obtained from the patients' records.

Statistical methods. The phagocytic activity (PA) (as defined above, page 10) in presence of a tested serum was always compared to that in a simultaneously run control serum. The results were expressed as the difference in PA between test serum (in duplicate) and control serum (in duplicate) ($\triangle$PA t-c). Provisionally 'low PA' in individual sera was said to be present if this difference was negative and outside the 95 per cent tolerance limit of the corresponding differences found in a standard population. At present, however, there is not enough information available for an assumption that PA in non-diseased populations are normally distributed. Furthermore, some of the other variables investigated may not have such a distribution. Hence, relations and differences were all analyzed by non-parametric statistical methods (Siegel 1956). Calculations of correlation coefficients were performed with the method of Spearman. The significance of differences between 2 samples was calculated with the median test, the Mann-Whitney U-test or with the Wilcoxon's matched-pairs signed-ranks test.

Comments on some serological and clinical features of the patient groups

SLE-group. The prevalence of ARA-criteria was comparable to that of studies reported by others (see Hughes 1977). The median Clq, C4 and C3-values were low compared to those of normal populations. Anti-nDNA were found in 45 per cent and cryoglobulins in 50 per cent

of sera tested. The ClqBA-test was positive in 75 per cent of the sera.

RA-group. Many patients had a severe disease evidenced by a high frequency of positive ANA-tests (49 %), of subcutaneous nodules (41 %), of vasculitis (20 %) and of amyloidosis (26 %). None of the patients had positive tests for anti-nDNA. 86 per cent were rheumatoid factor positive. The median level of C4 was low (82 %) compared to what is found in non-complicated RA with low to moderate disease activity (Franco & Schur 1971, Hunder & McDuffie 1973, Berglund et al. submitted for publication). On the other hand, Cls, C5 and factor B showed median values higher than those in normal populations. Cryoglobulins were detected in 51 per cent of 37 sera tested. The ClqBA-test was positive in 86 per cent of the 57 sera.

CTD-group. The largest subset in this group, the patients with SLE-like syndromes, had lower levels of Clq, C4 and C3 than the patients with other CTD. Cryoglobulins were only found in 21 per cent but the ClqBA-test was positive in 65 per cent of the sera. Only one serum in this group (from a patient with an SLE-like syndrome) contained anti-nDNA.

IJD-group. PA, glass adherence and cell morphology did not in any case differ from that of normal controls. Therefore, serological or other investigations were not performed in this group.

Impaired monocyte function in presence of rheumatic sera

Low phagocytic activity (PA) in some rheumatic diseases (II). In the sera from the SLE-group normal monocytes showed a significantly lower PA than in the sera from any of the other groups. Low PA was found in 51 per cent of 43 SLE-sera, 16 per cent of 57 RA-sera, 22 per cent of 18 SLE-like syndromes but in none of 16 other CTD-sera and 30 IJD-sera. The PA in the group of sera from patients with SS or MCTD did not differ from

patients without these additional syndromes.

Decreased bacterial killing in SLE (V). Of the 43 SLE-sera, 14 were randomly chosen for the bacterial killing experiments. The bactericidal activity of normal monocytes was impaired in the presence of 8 of these 14 sera.

Decreased glass adherence in SLE (IV). The number of glass adherent cells per monocyte culture (GAC) was determined in 40 of the 43 SLE-sera after incubation with yeast cells (YC) and compared with GAC in the simultaneously run control experiments. In SLE-sera the median GAC was significantly ($p<0.001$) lower than that in control sera.

Since addition of YC reduces GAC in monocyte cultures with normal sera (I), GAC in SLE-sera was also determined in preparations without YC. In 7 sera from patients with active SLE, GAC was always lower (median GAC 32 x 10^3) than in simultaneously run control sera (median GAC 113.5 x 10^3) ($p<0.01$).

Decreased spreading in SLE (IV). The spreading of monocytes in presence of selected sera from patients with active SLE was compared with that of simultaneously run control experiments in normal serum (no YC added). The results of 7 different experiments showed that in SLE-sera only 20-51 per cent of the monocytes were spread while in control sera 59-84 per cent were spread ($p<0.01$).

Comments

Low PA was found in about 50 per cent of 43 'consecutive' sera from patients with SLE. Thus, these findings are in agreement with previous findings of impaired human monocyte phagocytosis of yeast cells (Svensson 1975) and of antibody-coated sheep erythrocytes (Temple & Loewi 1977) in small numbers of patients with SLE. In addition, the present study has shown that low PA could be demonstrated in sera also from patients with rheumatoid arthritis and SLE-like syndromes but not in sera from other multisystem connective tissue diseases or

other inflammatory joint diseases. However, the number of patients in individual diagnostic groups was small and it cannot be excluded that low PA might not occur in investigations with larger patient populations.

In sera with low PA, the microscopical picture usually suggested decreased glass adherence and decreased spreading. As the prevalence of low PA, as mentioned, was the highest in SLE, glass adherence and spreading were studied only in SLE-sera. For the same reason the preliminary study on bacterial killing was also performed on SLE-sera. An additional argument for this choice was that increased susceptibility to bacterial infections is an important feature of SLE (Staples 1974).

Associations of deficient monocyte function with clinical or laboratory features of the disease

Low phagocytic activity (PA) and hypocomplementaemia (II and III). In most cases, low PA was associated with low levels of one or more complement components. In SLE-sera, significant correlations were found between PA and the complement components Clq, Cls, C4, properdin, C3 and C5 but not factor B. In RA,PA was significantly associated with low C4. In addition, PA was low in all of the few sera with decreased Clq or C3.

In the CTD-group PA was low in only 4 sera. Three of these had very low Clq, C3 and C4 levels.

Low PA and tests for circulating immune complexes (CIC) (II). In SLE the presence of cryoglobulins, indicating CIC, was associated with low PA ($p<0.01$). In RA-sera, in which PA was low, cryoglobulins were detected in all 6 sera tested but were also found in many cases without low PA. There was no significant association between low PA and presence of CIC as determined by the ClqBA-test.

Low PA and antinuclear antibodies (II and III). ANA were found in most sera associated with low PA. No asso-

ciation was established between anti-RNP and low PA. Anti-nDNA were found almost exclusively in SLE-sera. High titres for these antibodies were associated with low PA.

Disease manifestations and low PA (II and III). In SLE, low PA was significantly associated with a history of rash and cylindruria and presence of a bad general condition, arthritis, rash and nephritis at the time of the phagocytosis test.

All 4 sera in the CTD-group associated with low PA were obtained from patients with SLE-like syndromes with vasculitis. Two of these patients developed a severe disease with glomerulo-nephritis consistent with a diagnosis of SLE.

In RA the median PA was lower in sera from patients with vasculitis or Sjögren's syndrome but this was not statistically significant.

PA and some laboratory tests in SLE (III). PA correlated significantly with haemoglobin ($r = 0.51$, $p<0.01$) and ESR ($r = -0.58$, $p<0.001$). No associations were found between PA and white blood cell count, blood lymphocytes, serum IgG, serum IgA, serum orosomucoid, rheumatoid factor or CRP. CRP was markedly (>100 mg/l) increased in 3 patients, all suffering from infection at the time of investigation while this test was moderately (13-100 mg/l) increased in 11 patients, all without evidence of infection.

Clinical disease activity, PA and serological manifestations in SLE (III). It was shown that low PA correlated significantly with high clinical disease activity expressed as the clinical activity index (CA). Furthermore, from serial examinations of the same patients it was also found that PA improved as the disease activity declined. Similarly, the values for Clq, C3 and C4 increased as the disease activity declined. A tendency towards normalization was also noted for cryoglobulins and anti-nDNA but not for the ClqBA-test.

PA and ongoing prednisolone treatment (II and III). About half of the patients with SLE and RA were on cor-

ticosteroids at the time of investigation. There was no significant association between the level of PA and the presence or absence of corticosteroids. Nor did serial examinations of SLE-patients show any evidence of a direct influence of corticosteroid treatment on the outcome of PA.

Bacterial killing, PA and clinical status in SLE (V). The patients with impaired bacterial killing showed higher clinical disease activity estimated as CA and were more frequently on corticosteroid therapy than the patients with 'normal' bacterial killing. Furthermore, infections were more common during a 2-year follow-up period among patients with reduced bacterial killing.

The data did not suggest any differences as to PA or complement levels between the group with 'normal' and the group with reduced bacterial killing.

Glass adherence, PA and clinical disease activity in SLE (IV). Low GAC was associated with high clinical disease activity estimated as CA ($p<0.01$). Furthermore, GAC correlated with PA ($r = 0.60$, $p<0.001$). In these experiments GAC was estimated after addition of YC.

Spreading and clinical status in SLE (IV). In the light microscope the monocyte spreading in presence of serum from patients with active SLE and low PA appeared reduced compared to simultaneously run control preparations. Measurements of spreading in 7 such sera substantiated this observations (see above page 22).

All these sera showed evidence of circulating immune complexes (both cryoglobulins and positive ClqBA-tests) and were hypocomplementaemic. In one case, serial measurements of spreading were performed. It was shown that spreading normalized when disease activity, PA and complement levels improved.

Comments

Evidence of hypocomplementaemia was found in nearly all sera associated with low PA and was most frequent and pronounced in the SLE-group. Hypocomplementaemia in SLE

has been widely recognized (for review see Schur 1975). and has been found to correlate with high disease activity, especially with the presence of active glomerulo-nephritis. The mechanism behind hypocomplementaemia has mainly been ascribed to consumption by circulating immune complexes (CIC) although reduced synthesis of certain complement components has been reported in SLE (Petz et al. 1977). CIC in SLE are generally assumed to activate complement via the classical pathway (Lambert & Casali 1978). The significant correlations between PA on the one hand and Clq, Cls and C4 on the other may be indirect evidence for a possible role of CIC in the impairment of yeast cell phagocytosis (YCP).

In most cases impaired YCP was associated with evidence of CIC either as positive ClqBA-test or presence of serum cryoglobulins. The ClqBA-test, however, was also positive in many cases without low PA. In fact, this test was positive in 75 per cent of SLE-sera and in 86 per cent of RA-sera, which is very close to the 78 per cent and 83 per cent, respectively, obtained with the same method in a recent WHO-study (Lambert et al. 1978). As the ClqBA-test is positive in many cases with inactive disease this test also detects complexes that are of little significance for the development of systemic manifestations and immunological abnormalities in diseases like SLE and RA. However, in SLE, very high levels of Clq binding activity was often seen in sera associated with low PA although subsequent serial examinations of some of these cases showed persistance of high Clq binding activity in spite of improval of both PA and CA.

Cryoglobulinaemia was significantly associated with low PA in the SLE-group. Cryoglobulins are frequently found in SLE especially in severe disease, often with nephritis as first described by Christian et al. (1963). This was also the case in the present study (cryoglobulins were present in 7 of 10 sera from patients with ongoing nephritis). The present finding of a significant association of cryoglobulinaemia with low PA in

SLE is a further suggestion of a relation between low PA and presence of CIC.

In RA-sera associated with low PA cryoglobulins were detected in all of 6 sera tested. However, cryoglobulins were also found in several cases with 'normal' PA. Cryoglobulins are common in RA as shown in the present study and by others (Weisman & Zvaifler 1975) and do not only occur in severe disease with vasculitis but frequently also in uncomplicated cases (Weisman & Zvaifler 1975). In addition, cryoglobulins in RA have not, as in SLE, been associated with glomerulo-nephritis. This might suggest that different types of cryoglobulins occur in RA and that they may reflect CIC with properties different from those in SLE.

Anti-nDNA, assessed by the Crithidia luciliae-test, had high specificity for the diagnosis of SLE. Thus, in patients with diagnoses other than SLE anti-nDNA were only found in one other case,which had an SLE-like syndrome.

In agreement with the recent findings of Ballou and Kushner (1979), who used the same method, high anti-nDNA titres (≥1/100) were only found in sera from patients with high clinical disease activity, often with evidence of nephritis. In the present study, PA was usually low in presence of such sera. Serial examinations of some SLE-patients showed that the anti-nDNA titre decreased or became negative when disease activity declined.

Anti-RNP, detected in 14 sera, did not seem to influence the outcome of PA.

In the present study patients with reduced bacterial killing tended to have a higher frequency of infections during a 2-year follow-up period compared to those with normal killing capacity. One explanation to frequent occurrence of infections in SLE might be reduced bacterial killing by monocytes as demonstrated in the present study (V) and by granulocytes as reported by others (Jasin et al. 1974).

Corticosteroid therapy was more frequent among patients with reduced bacterial killing. However, the number of patients studied was small and the dosage of corticosteroids was low or moderate in all cases except one. Corticosteroids have been shown to reduce bacterial killing by monocytes (Rinehart et al. 1975) and by granulocytes (Mandell et al. 1970). A recent computer analysis of factors influencing frequencies of infections in SLE showed that corticosteroid therapy contributed to a high incidence of infections even if correction was made for the influence of disease activity (Gintzler et al. 1978). Thus, the increased frequency of infections in SLE may be related also to corticosteroid treatment.

3. Possible mechanisms behind impaired yeast cell phagocytosis

General considerations

Opsonic factors. An efficient phagocytosis of most virulent strains of bacteria and of yeast requires opsonization (Wilkinson 1976). Thus, yeast cells (YC) have to be opsonized by serum factors in order to be adequately phagocytosed by monocytes (I) or by neutrophils (Miller & Nilsson 1970). The major serum opsonic factors are IgG antibodies and complement components (Walters & Papadimitriou 1978). Opsonization of YC is possible with specific antibody and/or complement. Purified anti-yeast IgG from immunized rabbits promoted phagocytosis by neutrophils in the absence of serum (Hed 1979). In human sera specific antibodies do not seem to be a requisite for yeast cell phagocytosis (YCP). Thus, agglutination antibody to Baker's yeast were not detected in 50 sera exhibiting normal YCP by cultured neutrophilic granulocytes (Brandt 1967). Furthermore, in agammaglobulinaemic serum YCP was comparable to that in normal sera (Yamamura & Valdimarsson 1977).

The YC-wall contains insoluble polysaccharides with a potency to activate complement via the alternative pathway (Pillemer et al. 1954, Agnello 1978). This leads to the generation of C3b. Available evidence indicates that C3b, bound to the YC-surface, is the most important complement-derived opsonic factor for YCP (for review see I).

It seems likely that in human sera opsonization of YC is mainly a function of the alternative pathway. Specific antibodies on the YC could produce complement activation by the classical pathway. However, two immunofluorescent studies of YC after mixing with fresh human or animal sera showed C3 on all YC, IgM on 28 per cent of YC, while IgG was not found at all (Hed & Stendahl 1978, Kavai et al. 1979). Considering the role of the classical pathway it should be pointed out that classical pathway components may be required for efficient activation of the alternative pathway (Jackson et al. 1979).

Partical uptake. The uptake of opsonized particles and of immune complexes by mononuclear phagocytes is mediated by cell surface receptors for the Fc-part of the IgG molecule and for C3b and C3d (Huber et al. 1968, Ehlenberger & Nussenzweig 1977).

The relative roles of the IgG-receptor and the C3-receptors in the attachment and ingestion phases of the phagocytic process is controversial. According to Ehlenberger and Nussenzweig (1977) the C3-receptors are primarily involved in particle attachment while only the IgG-receptor is also able to promote ingestion. However, Cohn et al. (1978) have shown that also the C3-receptor may mediate ingestion provided that the macrophage is activated. Furthermore, Hed (1979) has found that if C3b-coated YC were incubated with the phagocytes for as long as 30 minutes they were ingested to the same extent as IgG-antibody-coated YC.
Experiments (VI). In the present study (III) impaired YCP was found in presence of about 50 per cent of all SLE-sera tested. Furthermore, low phagocytic activity (PA) was associated with high clinical disease activity, cryoglobulinaemia and hypocomplementaemia. The hypotheses considered so far is that low PA in some rheumatic sera is due to an interference of immune complexes with macrophage functions and/or to deficient opsonization of the YC.

For an exploration of these alternatives several experimental approaches are possible. A few of these have been investigated in this study. Thus, the following two types of experiments were performed:

1) Immune complex analogues added to monocyte cultures in normal serum
   a) Cryoglobulins (CG) were isolated from patients with SLE, RA and essential (Brouet et al. 1974) cryoglobulinaemia. These CG were added to cultures of normal monocytes in normal serum. PA was low in presence of CG from 7 of 7 patients with SLE or RA with vasculitis and from 2 of 5 patients with essential cryoglobulinaemia.

IgM and C3 were detected only in CG associated with low PA. Furthermore, low PA was only found with the CG isolated from hypocomplementaemic serum.

b) Human heat-aggregated IgG (HAG) was isolated by gel filtration on Sephadex G200. The results showed that addition of HAG ≥19S to the culture medium could reduce PA while no effect was obtained by the intermediary fractions (unpublished observations) or by the 7S fraction from the same HAG-preparations. Concentrations at or above 0.06 mg HAG ≥19S/ml of the serum part of the medium were necessary to obtain this effect which was observed after incubation for 2-24 hours. However, when the incubation time was extended to more than 3 days,PA showed a tendency to improve. In contrast to YCP, uptake of silica particles was not influenced by HAG. Replacement of the HAG-containing medium by fresh serum restored YCP.

2) Preopsonized YC added to monocyte cultures in SLE-serum or in serum with HAG

In some experiments the YC were opsonized with fresh normal serum before incubation with the monocytes. They were then added to cultures in which monocytes had been incubated with HAG or with SLE-serum. In these experiments phagocytosis was unaffected whereas, in parallel cultures, phagocytosis of not preopsonized YC was impaired.

Comments

In principle,two approaches can be used to study the possible influence of immune complexes in SLE-sera on yeast cell phagocytosis (YCP). One is to remove the immune complexes from the sera. However, reliable methods for the selective removal of immune complexes from serum are lacking. The use of ligands, such as solid phase C1q or staphylococcal protein A was considered to in-

volve important elements of uncertainty.

Following the other approach, immune complex analogues were added to normal serum. It was then shown that human heat aggregated IgG (HAG) or serum cryoglobulins (CG) from patients gave a reduction of PA comparable in magnitude to that observed in sera from active SLE.

The precise mechanism by which soluble CG, soluble HAG and sera from active SLE exerted their effect on YCP has not been clarified. Nor is the possibility excluded that HAG and CG, although regarded as immune complex analogues, may act differently from SLE-serum in the present assay system. However, the data obtained in the present study permit some considerations concerning the possible mechanisms involved.

A simple explanation to low PA would be that monocytes occupied by ingesting immune complexes might be incapable of handling an extra-load of added YC. However, this is not a likely explanation since a) the monocytes could attach and ingest silica particles in presence of HAG, b) when YC were preopsonized, adequate YCP was demonstrated in monocyte cultures with HAG or with SLE-serum, c) the results of kinetic studies on guinea-pig macrophages (unpublished data) suggest that the cells cannot be saturated by HAG even in concentrations much higher than those necessary for the impairment of YCP.

The possibility that the monocytes were permanently damaged by the HAG is unlikely since replacement of the HAG-containing medium by fresh serum restored the YC uptake of the monocytes.

Another possibility would be that immune complexes block monocyte Fc and/or complement receptors. However, the experiments with preopsonized YC showed that adequate phagocytosis was possible. This observations is evidence against cell surface receptor blockade. However, in some sera a considerable loss of glass adherence was observed after addition of preopsonized YC. Therefore, an attempt to validate these findings in a

larger series of experiments of this kind was not considered worthwhile.

The conclusion of these results and considerations is that if immune complexes play a role for the impairment of YCP it appears to be mediated by interference with the opsonization of YC.

Decreased serum complement components is a prominent feature in SLE(reviewed by Schur 1975). In the present study associations were found between hypocomplementaemia and low PA. The most simple explanation would therefore be that SLE-sera had too low levels of one or more complement components to support an adequate opsonization. It should be noted, though, that low PA was also present in some sera with only moderately decreased complement levels. However, the amounts of the individual complement components necessary for an adequate YCP are unknown. Dilution of serum decreases the efficiency of the alternative pathway (Schreiber et al. 1978) and this may make the assay system used more sensitive to hypocomplementaemia.

An additional possibility is that the limited supply of complement components from SLE-serum, due to the immune complexes, is further reduced during incubation in the culture medium prior to the addition of YC. A depletion of complement components necessary for opsonization is also suggested by the experiments with immune complex analogues. It is well established that substances like aggregated IgG activate the classical pathway and also produce conversion of factor B (Götze & Müller-Eberhard 1971, Lachmann & Hobart 1978). The findings in the present study suggest that among immune complexes of the cryoglobulin type, only those that are associated with evidence of complement activation interfere with the phagocytosis of YC. This is in agreement with a recent report that showed conversion of factor B by cryoglobulins isolated from patients with rheumatic diseases (Arroyave et al. 1978). Like most of the CG with effect on YCP in the present study, the cryoprecipitates with effect on factor B conversion contained

IgM and complement components while those without did not (Arroyave et al. 1978).

Depletion of complement components might not only be due to direct activation through immune complexes. Immune complexes in SLE-serum may be phagocytosed by the monocytes. During this process monocytes are activated and may release lysosomal enzymes which may contribute to complement consumption by direct cleavage of factors such as C3 (Schorlemmer & Allison 1976). Thus, immune complexes might in various ways deprive the culture medium of complement components necessary for YC opsonization.

Macrophages in cultures have previously been shown to synthesize several complement components (Bentley et al. 1978, Lai A Fat & van Furth 1975). Synthesis of complement components might accordingly explain the present findings of improved phagocytosis after prolonged incubation although it is possible that maturation of monocytes to macrophages had contributed to the increased phagocytic capacity (I).

The present investigation gave evidence for the possibility that the impairment of YCP found in many SLE-sera may be related to the presence of immune complexes. Furthermore, it was suggested that the effect might be due to <u>in vivo</u> and/or <u>in vitro</u> depletion of complement components necessary for opsonization of the yeast cells in the monocyte culture.

GENERAL DISCUSSION

The present study has shown that glass adherence (IV), spreading (IV), bacterial killing (V) and yeast cell phagocytosis (YCP) (II) were often deficient in a number of sera from patients with SLE (III). Impaired YCP was also found in some sera from patients with SLE-like syndromes and rheumatoid arthritis (II). Alterations of monocyte membrane function and deficiency of opsonization of the phagocytic particles were suggested as the most likely mechanisms behind the findings.

Glass adherence has been considered as 'attempted phagocytosis' (Territo & Cline 1977), i.e. an attempt of the cell to phagocytose the glass surface on which it resides. This view is seemingly supported by the findings in the present study that decreased glass adherence was significantly associated with low phagocytic activity (PA) (IV). However, preopsonized YC could be adequately phagocytosed in monocyte cultures expressing decreased glass adherence (VI). Therefore, the process involved in glass adherence may not be identical with that responsible for the uptake of opsonized particles.

Mononuclear phagocytes perform locomotion by crawling on surfaces. Adhesion to a substrate has been considered to be a pre-requisite for and spreading an expression of locomotion (Stossel 1978). Glass adherence and spreading might accordingly be looked upon as different phases of the same cellular processes,which involve the cytosceletal structures, i.e. microtubules and microfilaments (Cheung et al. 1978). In the present study, deficient glass adherence and spreading were in

most cases parallel phenomena observed in heat-inactivated normal sera, SLE-sera and in fresh normal sera to which heat aggregated IgG (HAG) had been added. When glass adherence and spreading are impaired, mononuclear cells would be expected to show decreased random movement and deficient response to chemotactic stimuli. Preliminary studies in this laboratory with time-lapse-technique have shown decreased random mobility of normal monocytes in serum from a patient with SLE (unpublished observations). The question whether mononuclear phagocytes in pathological sera give inadequate chemotactic responses has so far not been answered.

Recent reports suggest that complement components are essential for spreading. Thus, factor B in its inactivated form was found to be an important serum factor to provide an adequate monocyte spreading (Götze et al. 1979). This finding is compatible with the observation made in the present study that reduced spreading was observed in complement inactivated (by heat inactivation) serum (IV). In pathological sera reduced spreading might be due to immune complexes that deplete the culture medium of complement components necessary for normal spreading.

Reduction of spreading due to binding of immune complexes to the Fc (but not the C3b) receptors of peritoneal macrophages have been described by Douglas (1976) who suggests that interference of immune complexes with the macrophage membrane might influence spreading. Thus, it is not clear if the impaired glass adherence and spreading were due to depletion of complement and/or to a direct influence of immune complexes on the monocyte.

Defective Fc-receptor function <u>in vivo</u> of splenic macrophages has recently been described in patients with SLE with circulating immune complexes (Frank et al. 1979). The findings of decreased glass adherence and spreading in SLE-sera associated with low PA might reflect a disturbance of monocyte membrane function. If so, these observations may have a bearing on

the interpretation of impaired phagocytic function in SLE and, in addition, rasie the question whether other membrane functions are disturbed by this alteration of the cell membrane.

Measurement of glass adherence and spreading have been proposed as aids in the evaluation of monocyte function (Territo & Cline 1978). The results of the present study indicate that these methods can be used for the detection of serum dependent impairment of these in vitro monocyte functions. However, in their present shapes, the methods are time-consuming and laborious and need further development before they can be recommended for clinical routine use.

Monocyte bacterial killing was deficient in 8 out of 14 SLE-sera. The data suggested that impaired killing was more common among patients with an increased frequency of infections during a follow-up period. If these preliminary observations will turn out to be true, testing bacterial killing might be a useful aid to deliniate a subgroup of patients with SLE having increased susceptibility to infections. Since also defective granulocyte bacterial killing in SLE has been reported (Jasin et al. 1974) we now intend to extend our studies to a parallel investigation of granulocyte and monocyte bacterial killing in sera from patients with inflammatory rheumatic diseases.

The yeast cell phagocytosis test measures the influence of serum on the capacity of normal monocytes to attach and ingest opsonized particles - a most important function in the host defence against antigenic challenge (Wilkinson 1977).

A normal outcome of the test provides the information that, in the serum tested, complement activation to C3b is sufficient for opsonization to occur, and that monocyte membrane function (including the C-receptors) is adequate for attachment and ingestion of the particles. Accordingly, low PA indicates a deficiency either of opsonization or of membrane function. The present data suggest that low PA in SLE-sera reflects

the presence of immune complexes that in some way produce hypocomplementaemia resulting in inefficient opsonization. Studies are now in progress to elucidate whether this may be true and, if so, which complement components are lowered and to what extent.

In SLE-sera low PA was associated with high clinical disease activity, hypocomplementaemia and cryoglobulinaemia suggesting that YCP reflects the inflammatory process in SLE. Very low, almost abolished YCP was present in all sera from seriously ill patients with SLE (III). After treatment, these abnormalities showed a clear tendency to disappear as the patient improved.

Falling levels of complement (Schur 1975), changing anti-nDNA titres (Hughes 1975) or rising levels of CIC (Lambert & Casali 1978) are not always reliable indicators of impending flare of SLE. Therefore, it seems to be of interest to perform longitudinal studies of YCP in SLE to investigate the possibility suggested by preliminary observations in this laboratory that a falling PA may herald an exacerbation of the disease and, conversely,that a rising PA may be an early indicator of declining activity of the disease.

If any of these possibilities should prove to be true the YCP-test has a potential as a guide for the therapeutic management of SLE. An important task is then to investigate whether the test can be applied to clinical routine use.

The first step would be to determine the distribution of PA among normal sera from a larger population. If the distribution proves to be normal - as suggested by the present study - calculation of tolerance (confidence) limits for normal PA is enabled. This would then provide a basis for the identification of abnormal PA in individual pathological sera. The use of a standard pool of sera as 'control serum' would probable increase the accuracy of the method.

In its present shape and in the hands of an experienced technician, the YCP-test can be applied to as many as 15-20 sera in one day. However, as the method

requires cell separation facilities and as the determination of PA is time consuming, modifications of the method should be considered. Thus, the use of purified macrophages from laboratory animals might make cell separation unnecessary. Rapid and accurate measurement of phagocytosis might be accomplished by radiolabelling of the phagocytic material which might have the additional advantage of increasing the span of the method. Furthermore, particles with similar opsonic requirements for phagocytosis might prove more appropriate than yeast cells.

SUMMARY

In an earlier pilot study it was shown that yeast cell phagocytosis by normal human macrophages was impaired in presence of SLE-sera. These findings lead to the present investigation in which several *in vitro* functions of normal human monocytes were studied in presence of sera from patients with inflammatory rheumatic diseases.

A method for the study of the occurrence and variation of decreased serum dependent phagocytosis was developed. Human mononuclear cells were separated in an Isopaque-Ficoll gradient and incubated in a medium with serum in Parker 199. Heat-killed,not preopsonized yeast cells were added for phagocytosis. The phagocytic activity (PA) was expressed as the median number of yeast cells ingested per monocyte in 100 monocytes. The results of testing various modifications of this method indicate that an appropriate procedure, allowing a sufficient span for measuring 'subnormal' phagocytosis, is incubation of the monocytes in 15 per cent serum for 120 minutes followed by a further incubation with 10^7 yeast cells for 30 minutes. Furthermore, sources of experimental variation of PA in normal sera were investigated. The evaluation of PA in a test serum was standardized by subtraction with the PA of a simultaneously run control in normal serum.

Monocyte yeast cell phagocytosis was studied in presence of sera from 164 patients with different inflammatory rheumatic diseases. The results showed that low PA was found in 51 per cent of 43 sera from patients with SLE, 16 per cent of 57 sera from patients with RA,

22 per cent of 18 patients with SLE-like syndromes but in none of 16 patients with other inflammatory connective tissue diseases and 30 patients with inflammatory joint diseases other than RA.

Other in vitro monocyte functions were only studied in sera from patients with SLE. The bactericidal activity of normal monocytes was impaired in the presence of 8 of 14 randomly chosen SLE-sera. Glass adherence was significantly lower in SLE-sera than in control sera. The spreading of monocytes in sera from patients with active SLE was significantly reduced compared to that in normal sera.

Relations between impaired phagocytic activity and clinical or laboratory features of the disease were studied. Low PA was associated with

- low levels of one or more complement components
- the presence of cryoglobulins in SLE-sera but not in RA-sera
- high titres of anti-nDNA antibodies
- high clinical disease activity in SLE (using a clinical activity index free from immunological data)
- a history of rash or cylindruria
- presence of bad general condition, arthritis, rash or nephritis at the time of phagocytosis test.

Patients with impaired bacterial killing showed higher clinical disease activity, tended to be more frequently on corticosteroids and had more clinically relevant infections during a follow-up period than patients with normal bacterial killing.

Decreased glass adherence was associated with high clinical disease activity and with low PA.

Decreased spreading was only observed in sera from patients with active SLE, all having evidence of circulating immune complexes and hypocomplementaemia.

Possible mechanisms behind impaired yeast cell phagocytosis were investigated in a preliminary fashion in two types of experiments:

1) Immune complex analogues were added to monocyte

cultures in normal serum

a) Cryoglobulins (CG) isolated from patients with SLE, RA and essential cryoglobulinaemia. The results showed that PA was low in presence only of CG containing IgM and C3 and originating from hypocomplementaemic sera.

b) Human heat aggregated IgG (HAG). Addition of HAG ≥19S to the culture medium could reduce PA while this effect was not obtained with the intermediary fractions (unpublished observations) or with the 7S fractions from the same preparations.

2) Preopsonized yeast cells were added to cultures in which monocytes had been incubated with HAG or with SLE-serum. In these experiments phagocytosis was unaffected, whereas, in parallel cultures, phagocytosis of not preopsonized YC was impaired.

In conclusion, these experiments and the clinical studies gave evidence for the possibility that impairment of yeast cell phagocytosis found in many SLE-sera may be related to the presence of immune complexes. Furthermore, it was suggested that the effect might be due to in vivo and/or in vitro depletion of complement components necessary for opsonization of the yeast cells in the monocyte culture. The impairment of glass adherence and spreading in presence of sera from patients with active SLE may have a similar mechanism.

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